

Spontaneous and induced gynogenesis in sterlet *Acipenser ruthenus* Brandt

FOPP-BAYAT DOROTA* and WOZNICKI PAWEŁ

University of Warmia and Mazury in Olsztyn, Department of Ichthyology, 10-957 Olsztyn, Poland.

Abstract — Spontaneous diploidization of the maternal chromosome set (SDM) in *Acipenser ruthenus* was described. The gynogenetic progeny of sterlet (*Acipenser ruthenus*) were produced by the egg fertilization of UV-irradiated sperm of bester (*Huso huso* x *A. ruthenus*). The microsatellite DNA analysis and chromosome counting was applied for identification of gynogenetic progeny of sterlet.

Key words: *Acipenser ruthenus*, chromosomes, gynogenesis, microsatellites, sterlet.

INTRODUCTION

Spontaneous diploidization of the maternal chromosome set (SDM) is a well-known natural event in fish. This event was described in some species of teleost fishes, for example in: *Oncorhynchus mykiss* (THORGAARD and GALL 1979), *Pleuronectes platessa* (THOMPSON *et al.* 1981), *Tinca tinca* (FLAJSHANS *et al.* 1993), *Cyprinus carpio* (CHERFAS *et al.* 1991; 1995), *Cobitis biwae* (KUSUNOKI *et al.* 1994) and *Oreochromis niloticus* (EZAZ *et al.* 2004). VAN EENENNAAM *et al.* (1996) noticed the viability of few individuals in a haploid group of *Acipenser transmontanus*. There was the only case of that event in *Acipenseridae*. Even though, the normal-appearing fish without any paternal inheritance could be due to SDMs, the cytogenetical confirmation of the diploid status had not been provided in those fish.

Induced gynogenesis is one of the main methods of genomic engineering applied in fishes (ARAI 2001). This method is especially attractive for studying the sex determination mechanism in sturgeons and for production of all-female progeny of that fishes (MIMS *et al.* 1997; DEVLIN and NAGAHAMA 2002). All-female stocks of sturgeons could be also very useful for the farmed black caviar production despite the decline of natural populations of the sturgeon fishes.

The phenomenon of SDM presence could be detected in artificial gynogenesis experiments by the appearance of normal diploid progeny among the haploid control group in which no diploidization treatment has been applied (EZAZ *et al.* 2004).

The purpose of the present study was to induce the meiotic gynogenesis in sterlet using the optimal parameters of gynogenesis in this species (FOPP-BAYAT, unpublished). This study reports for the first time the SDM in *Acipenser ruthenus*, an event that was observed in the haploid control group in which eggs had been fertilized using UV-irradiated sperm during experiments on gynogenesis induction.

MATERIALS AND METHODS

Fish used for the study were progeny of broodstock maintained at the fish farm Wasosze near Konin, Poland. The eggs from one female of sterlet were fertilized by UV-irradiated heterologous sperm of bester (a semi-heterologous sperm from the hybrid *Huso huso* x *Acipenser ruthenus*). For UV-irradiation 1 ml of sperm was diluted with 9 ml of seminal fluid (supernatant from surplus semen centrifuged at 8000 rpm for 15min) and put into Petri dishes (Ø 100 mm) to a depth of approximately 1mm. These dishes were placed on a gently rotating platform (90 rpm) 50 cm below UV lamp (Philips 15W). Sperm was treated with UV irradiation for 70 seconds. After irradiation 10 ml of 15°C water from the

* Corresponding author: phone ++48 89 5234772; fax ++48 89 5233754; e-mail: foppik@uwm.edu.pl

incubation system was added to the irradiated sperm suspension, and this mixture was immediately added to ova.

Eggs were divided into two groups (~4000 eggs), held in individual (2 liters of volume) beakers and fertilized with 10 ml of diluted irradiated sperm at 15°C. The half of eggs was not heat shocked. Eggs to be heat shocked were transferred into separate box with perforated mesh and kept in water at 34°C for 2 min. Heat shocks were applied 18 min after fertilization. Fertilized eggs were placed in the Weiss incubators at 15°C. Surviving fry were kept in the 5-liter aquaria. Fry were fed with an artificial food for sturgeons.

All larvae with the normal appearance from the haploid group and 30 randomly-selected larvae from the heat-shocked group were sampled at the 7th day after hatching and stored in 96% ethanol. Fin clips from 2 parental individuals: female of sterlet, and male of bester were stored in 96% ethanol. Genomic DNA for two microsatellite loci: [*Afu-19* (MAY *et al.* 1997) and *Aox-45* (KING *et al.* 2001)] amplification was extracted using Chelex 100 method (WALSH *et al.* 1991). Aliquots containing PCR products and reaction buffer were electrophoresed using 6% polyacrylamide gel, and DNA bands were visualized by the silver staining method (TEGELSTRÖM 1986). Amplified fragments were sized by comparing with two DNA standards: ϕ X 174 DNA/*Hinf* I DNA Step Ladder (Promega) and 25bp DNA Step Ladder (Promega).

Chromosomes were prepared according to WOZNICKI *et al.* (1998) with modifications (the gill epithelium was used instead of the head kidney). Metaphase plates were observed by the Nikon Optiphot-2 microscope, photographed by Coolpix 995 digital camera and counted in Multiscan software. At least 5 well-spread metaphase plates from each specimen were analyzed.

RESULTS AND DISCUSSION

The survival rate in the shocked group was 21%. Cytogenetical and molecular analyzes showed that all 30 studied individuals were diploid gynogens without the paternal inheritance. Surprisingly, twenty six larvae from the haploid group survived and started to feed the exogenous food. The shape of those fish was normal without any signs of the "haploid syndrome" which was observed in almost all hatched larvae of sterlet in the unshocked group. All those larvae possessed

a diploid chromosome complement with ~118 chromosomes (typical for *Acipenser ruthenus*) (Fig. 1).

Two diagnostic microsatellite loci (*Afu-19* and *Aox-45*) analysis showed the lack of the paternal alleles in all studied larvae (Fig. 2 and 3, respectively). The same results of genetic analysis were obtained in the sample of 30 larvae from the heat-shocked group. These facts indicate that the normally shaped larvae in the haploid group must be the spontaneous diploid gynogens of *Acipenser ruthenus*.

The occurrence of such spontaneous normal diploid embryos among gynogenetic haploids, produced after fertilization of intact eggs with genetically inactivated sperm, is extremely rare (CHERFAS *et al.* 1995). SDM progeny from eggs fertilized with irradiated sperm can be identified in the same way as induced gynogenetic progeny, using techniques that can verify maternal inheritance, for example: phenotypic (GALBUSERA *et al.* 2000), biochemical, molecular and cytogenetical markers (EZAZ *et al.* 2004). In the present study the microsatellite DNA analysis and the chromosome number counting were applied for identification of spontaneous gynogens in sterlet.

The mechanisms of the SDM induction were studied by some authors (THOMPSON *et al.* 1981; FLAJSHANS *et al.* 1993; CHERFAS *et al.* 1995). CHERFAS *et al.* (1995) described three types of cytologi-

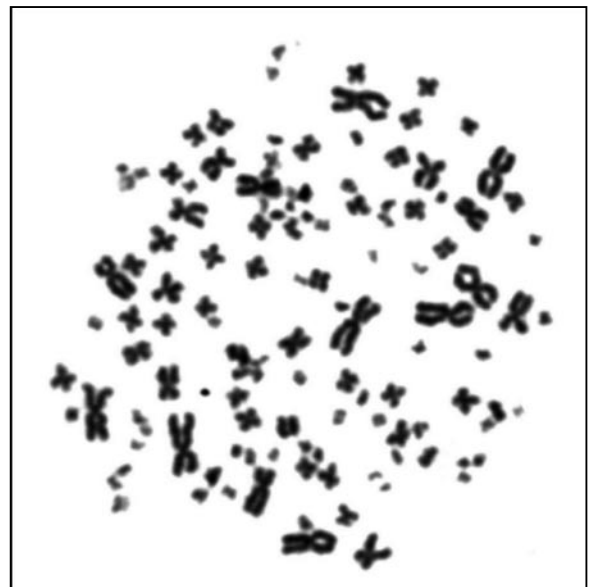


Fig. 1 — Chromosome plate from the spontaneous diploid *Acipenser ruthenus* ($2n = 118$).

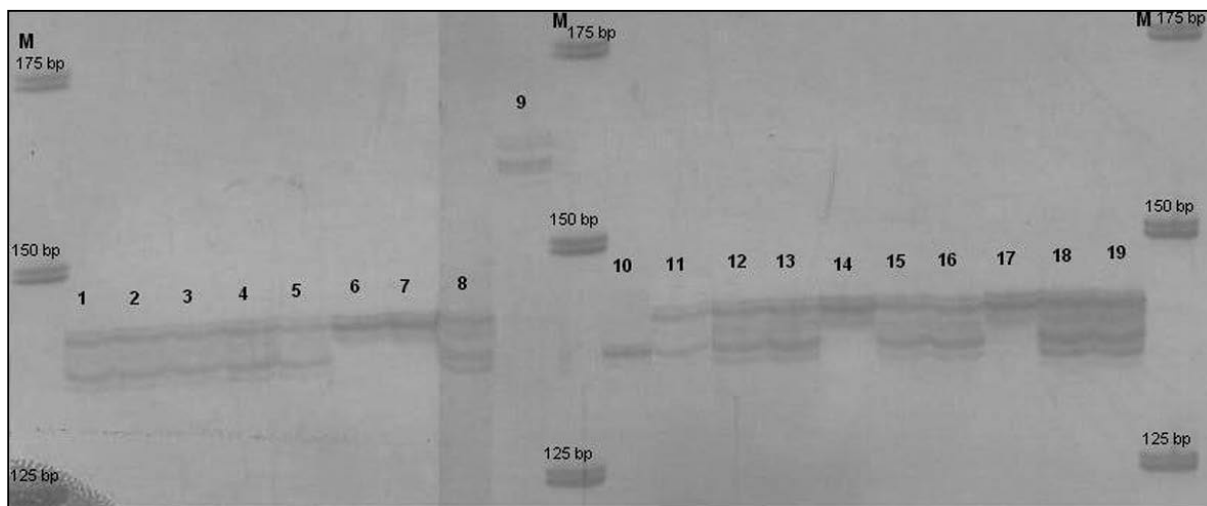


Fig. 2 — *Afu-19* alleles segregation in spontaneous diploid sterlet (*Acipenser ruthenus*). M = marker 25 bp (Promega). Sample 8: female of sterlet; sample 9: male of beluga x sterlet; samples: 1-7 and 10-19: gynogenetic offspring.

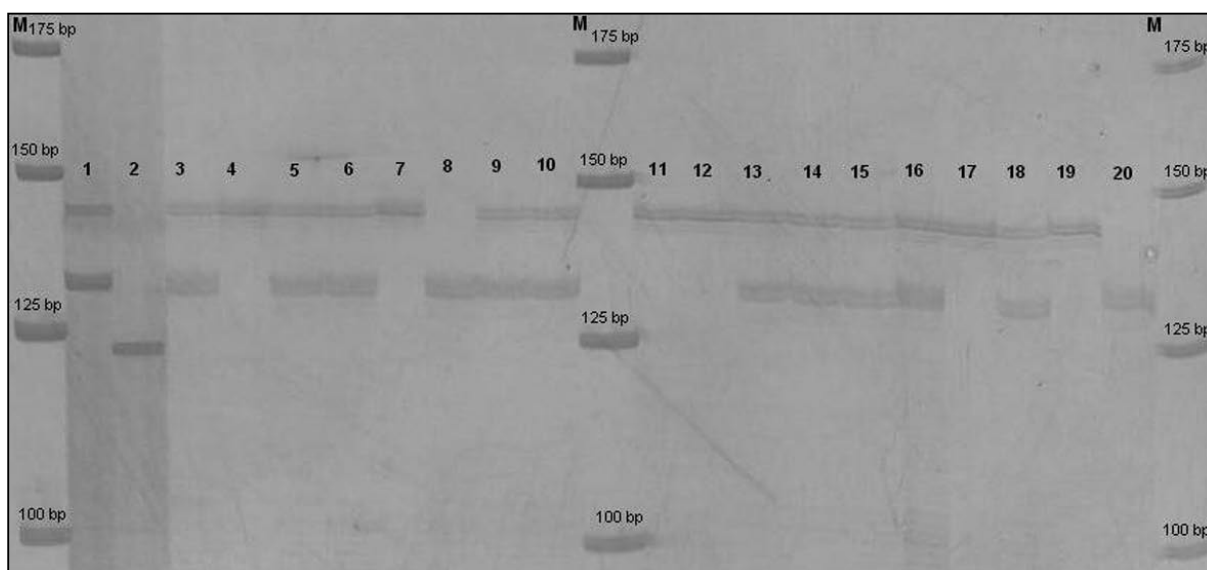


Fig. 3 — *Aox-45* alleles segregation in spontaneous diploid sterlet (*Acipenser ruthenus*). M = marker 25 bp (Promega). Sample 1: female of sterlet; sample 2: male of beluga x sterlet; samples 3-20: gynogenetic offspring.

cal transformations producing SDM in fish: pre-meiotic endoreduplication of the chromosome set, suppression of the first meiotic division and suppression of the second meiotic division. The most common cause of the SDM phenomenon is the suppression of the second meiotic division (EZAZ *et al.* 2004).

The present paper is the first well documented case of spontaneous gynogenesis in the species from family Acipenseridae.

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